# 2 OIPE

RAW SEQUENCE LISTING

PATENT APPLICATION: US/09/901,419

DATE: 07/24/2001 TIME: 10:53:13

Input Set : A:\Umo15311.app 3 <110> APPLICANT: The Curators of the University of Missouri

Output Set: N:\CRF3\07242001\I901419.raw

## **ENTERED**

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5 <120> TITLE OF INVENTION: LARGE SCALE EXPRESSION AND PURIFICATION OF RECOMBINANT
             PROTEINS
      8 <130> FILE REFERENCE: UMO1531.1
C--> 10 <140> CURRENT APPLICATION NUMBER: US/09/901,419
C--> 11 <141> CURRENT FILING DATE: 2001-07-09
     13 <150> PRIOR APPLICATION NUMBER: 60/218,125
     14 <151> PRIOR FILING DATE: 2000-01-13
     16 <160> NUMBER OF SEQ ID NOS: 2
     18 <170> SOFTWARE: Patentin Ver. 2.1
     20 <210> SEO ID NO: 1
     21 <211> LENGTH: 4087
    22 <212> TYPE: DNA
    23 <213> ORGANISM: Bos taurus
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    27 <222> LOCATION: (268)..(3180)
    29 <220> FEATURE:
    30 <221> NAME/KEY: sig_peptide
     31 <222> LOCATION: (268)..(363)
     33 <220> FEATURE:
     34 <221> NAME/KEY: misc_feature
    35 <222> LOCATION: (3178)
    36 <223> OTHER INFORMATION: A Poly (H) affinity tag comprising 6 His residues
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              have been inserted at the C-Terminus end of the
             coding region of the protein
    40 <400> SEQUENCE: 1
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    43 ggccagctca accagagctg ccactgatct tccacactta agcaaaccac accagtgagt 120
    45 ggcgaacatc aactcgtgct tgaaaaatac caacttggag cccggtttga gaagctacat 180
    47 cagagteteg agatgegacg etacaatetg cagtttteac tagetteeca gtaggttggg 240
    49 acagettggaa etetgecatt geecage atg etg eag tte agt etg tea eee ace 294
    50
                                      Met Leu Gln Phe Ser Leu Ser Pro Thr
    51
    53 ttq tcq atq qqa ttt cac qtq ata qcc atq qtq qct ctc ttq ttt tcc
    54 Leu Ser Met Gly Phe His Val Ile Ala Met Val Ala Leu Leu Phe Ser
                            15
                                                 20
    57 cat qtg gac cat ata agt gct gag aca gaa atg gaa gga gaa ggc aac
                                                                          390
    58 His Val Asp His Ile Ser Ala Glu Thr Glu Met Glu Gly Glu Gly Asn
    59
                        30
                                             35
    61 gag act ggc gag tgt act ggc tcc tat tac tgt aag aag ggg gtg att
                                                                          438
    62 Glu Thr Gly Glu Cys Thr Gly Ser Tyr Tyr Cys Lys Lys Gly Val Ile
                    45
                                         50
    65 tta ccc att tgg gag ccc cag gac cct tcc ttt gga gac aaa att gct
                                                                          486
    66 Leu Pro Ile Trp Glu Pro Gln Asp Pro Ser Phe Gly Asp Lys Ile Ala
    67
                                     65
    69 aga qcg act gtg tat ttt gtg gcc atg gtc tac atg ttt ctt gga gtc
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### Input Set : A:\Umo15311.app Output Set: N:\CRF3\07242001\I901419.raw

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	tca		att	act	gac	caa		atq	tee	tct	ata		qtc	atc	acq	tct	582
					Asp												
75	90					95					100					105	
		qaq	aaa	gaa	atc	acc	ata	aaq	aaa	ccc	aat	qqa	gag	acc	acc	aag	630
					Ile												
79			-		110					115					120		
81	aca	act	gtg	agg	atc	tgg	aat	gag	aca	gtg	tcc	aac	ctg	acc	ttg	atg	678
					Ile												
83				125					130					135			
					tca												726
86	Ala	Leu	Gly	Ser	Ser	Ala	Pro	Glu	Ile	Leu	Leu	ser		Ile	Glu	Val	
87			140					145					150				
89	tgt	ggc	cat	aac	ttc	act	gca	gga	gac	ctt	ggc	cct	agc	acc	atc	gtg	774
90	Cys	Gly	His	Asn	Phe	Thr	Ala	Gly	Asp	Leu	Gly	Pro	Ser	Thr	Ile	Val	
91		155					160					165					
					ttc												822
		ser	Ala	Ala	Phe		Met	Phe	Ile	Ile		Ala	Leu	Cys	Val		
	170					175					180					185	070
					ggg												870
	Val	Val	Pro	Asp	Gly	Glu	Thr	Arg	Lys		Lys	HIS	Leu	Arg		Рпе	
99					190					195					200		918
																: atc	910
102		· val	TIII	205		TIF	, ser	. 116	210		1 1 1 1	. 1111	115	215		. IIE	
		++0	. +a+			+ 00	, aat					r ata	t do			ttg	966
																Leu	200
107		. DCC	220		. ocz	001		225		. ,	. 010		230		,,		
		act			ttc	ttc	e ccc			att	ato	r t.t.t	act	: tac	ato	gca	1014
																Ala	
111		235					240		-			245		-			
113	gac	agg	agg	ctt	ctg	ttt	: tac	aac	tat	gto	tac	aag	agg	tat	. egg	gct	1062
																Ala	
115	250					255	5				260	)				265	
																tct	1110
118	Gly	Lys	Glr	Arg	Gly	Met	: 11e	: Ile	: Glu	His	Glu	Gly	Asp	Arg		Ser	
119					270					275					280		
121	tcc	aag	, aca	gaa	att	gaa	ato	, gat	ggg	aaa	gtg	gto	aat	tec	cat	gtt	1158
		Lys	Thr			Glu	ι <b>M</b> et	Asp			val	. Val	Asn			Val	
123				285					290					295			
																gac	1206
		Ser			Asp	Gly	/ Ala			. Le	Glu	val			ı Arg	Asp	
127			300					305					310				1054
																gaa	1254
				Glu	Glu	Ala			Glu	Met	Ala			ьтеп	ггуз	Glu	
131		315					320					325		2+-		++-	1302
																tta Leu	1302
134	ren	гАз	GLI	гъуs	nls	PIC	) GIU	гтув	GIU	1 116	: GIU	GI	пеп	TIE	: 016	пеп	

### RAW SEQUENCE LISTING

DATE: 07/24/2001 PATENT APPLICATION: US/09/901,419 TIME: 10:53:13

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138	Ála	Asn	Tyr	Gln	Val	Leu	Ser	Gln	Gln	Gln	Lys	Ser	Arg	Ala	Phe	Tyr	
139			-		350					355	-				360		
141	cgt	att	caa	gct	acc	cgc	ctg	atg	acc	gga	gca	ggc	aac	att	tta	aag	1398
		Ile															
143	-			365		-			370			_		375		-	
145	agg	cat	qca	qca	gac	caa	qcc	agg	aaa	gct	gtc	agc	atg	cat	gag	gtc	1446
		His															
147			380		-			385	•				390				
149	aac	acq	qaa	ata	qct	qaa	aat	qac	cct	qtc	agt	aaq	atc	ttc	ttt	gaa	1494
		Thr															
151		395					400	•				405					
153	caa	ggg	aca	tat	caq	tqt	ctq	gag	aac	tgt	qqc	aca	qta	qcc	ctg	acc	1542
		Gly															
155	410	-		•		415					420					425	
157	att	atc	cqc	aga	qqt	ggt	gat	ttg	acc	aac	act	gtg	ttt	gtt	gac	ttc	1590
		Ile															
159			-		430	-				435					440		
161	aga	aca	gag	gat	qqc	aca	qcc	aat	gct	gga	tct	gat	tac	gaa	ttt	acc	1638
162	Arg	Thr	Glu	Asp	Gly	Thr	Āla	Asn	Ala	Gly	Ser	Asp	Tyr	Glu	Phe	Thr	
163				445					450					455			
165	gaa	gga	act	gtg	gtc	ttt	aag	cct	ggt	gag	acc	cag	aag	gaa	atc	aga	1686
166	Glu	Gly	Thr	Val	va1	Phe	Lys	Pro	Gly	Glu	Thr	Gln	Lys	Glu	Ile	Arg	
167			460					465					470				
169	gtt	ggc	atc	att	gat	gat	gac	atc	ttt	gag	gag	gat	gag	aat	ttc	ctt	1734
170	Val	G1y	Ile	Ile	Asp	Asp	Asp	Ile	Phe	Glu	Glu	Asp	Glu	Asn	Phe	Leu	
171		475					480					485					
173	gtg	cat	ctc	agc	aac	gtc	aaa	gta	tct	ttg	gaa	gcc	tcg	gaa	gac	ggc	1782
174	Val	His	Leu	Ser	Asn	Val	Lys	Val	Ser	Leu	Glu	Ala	Ser	Glu	Asp	Gly	
175	490					495					500					505	
177	atc	ctg	gaa	gcc	agt	cat	gtc	tct	acc	ctt	gct	tgc	ctg	gga	tcc	ccc	1830
	Ile	Leu	Glu	Ala		His	Val	Ser	Thr		Ala	Cys	Leu	Gly		Pro	
179					510					515					520		
		act															1878
	Ser	Thr	Ala		Val	Thr	Ile	Phe		Asp	Asp	His	Ala		Ile	Phe	
183				525					530					535			
		ttt															1926
	Thr	Phe		Glu	Pro	Val	Thr		Val	Ser	Glu	Ser		Gly	Ile	Met	
187			540					545					550				
		gtg															1974
	Glu	Val	Lys	Val	Leu	Arg		Ser	Gly	Ala	Arg		Asn	Val	He	Val	
191		555					560					565					
		tat															2022
		Tyr	Lys	Thr	Ile		GLY	Thr	Ala	Arg		GLY	GŢĀ	GLu	Asp		
195						575					580					585	0070
		gac															2070
	Glu	Asp	Thr	Cys		Glu	Leu	Glu	Phe		Asn	Asp	GIu	ııe		гàг	
199					590					595					600		

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201	aca	ata	tca	gtc	aag	gta	att	gat	gat	gag Glu	gag	tat	gag	aaa	aac	aag	2118
202	THE	TTE	Ser	605	цуѕ	vaı	116	мэр	610	GIU	GIU	ıyı	GIU	615	MSII	пуз	
205										cgc							2166
	Thr	Phe		Leu	Glu	Ile	Gly		Pro	Arg	Leu	Val		Met	Ser	Glu	
207			620	-4-				625					630			~~~	2214
209	aag	Ture	gcc	CEG	LEA	LEG	Agn	Glu	Len	ggt Gly	G1 v	Phe	Thr	Tle	Thr	Glv	2214
211	цуз	635	AIG	ьси	пси	БСи	640	oru	Leu	017	011	645				027	
213										agg							2262
214	Lys	Tyr	Leu	Tyr	Gly		Pro	Val	Phe	Arg		Val	His	Ala	Arg		
	650					655					660					665	2210
217	cat	cca	CLC	CCC	Cor	act	ata	atc	mbr	atc Ile	gca	gat	Clu	Tur	yaı.	gac Aen	2310
219	HIS	PIO	Leu	PIO	670	1111	He	rre	1111	675	Ала	мэр	GIU	1 1 1	680	nap	
	aaq	caq	cca	ctq		age	aaa	qaq	gag	gaa	gag	agg	cgc	att	gcg	gaa	2358
222	Lys	Gln	Pro	Leu	Thr	ser	Lys	Glu	Glu	Glu	Glu	Arg	Arg		Ala	Glu	
223				685					690					695			
225	atg	ggg	cgc	ccc	att	ctg	gga	gag	cac	acc	aga	ctg	gag	gtg	atc	att	2406
226	Met	GIY	700	Pro	11e	Leu	GIĀ	705	HIS	Thr	Arg	Leu	710	vaı	ire	116	
	паа	σаа		tac	σασ	ttc	ааσ	,	acc	gtg	σac	aaa		att	aag	aaq	2454
										Val							
231		715					720					725					
										aac							2502
		Asn	Leu	Ala	Leu		Val	Gly	Thr	Asn	Ser	Trp	Arg	Glu	Gln	Phe 745	
235		~~~	a0a	2+0	201	735	a ort	act	aaa	gaa		aa c	gat	gac	gac		2550
										Glu							2550
239					750				1	755		-	•		760		
241	tgt	ggg	gag	gag	aag	ctg	ccc	tcc	tgt	ttt	gac	tac	gtg	atg	cac	ttt	2598
	Cys	Gly	Glu		Lys	Leu	Pro	Ser		Phe	Asp	Tyr	Val		His	Phe	
243	ata	nat.	a+a	765	+~~	226	at a	ata	770	000	+++	ata	000	775	202	nan	2646
245	CLG	Thr	Val	Dhe	Tro	L.ve	Val	Len	Phe	gcc Ala	Phe	Val	Pro	Pro	Thr	Glu	2040
247	пси	1111	780	Line	110	2,5	,	785					790				
249	tac	tgg	aac	ggc	tgg	gcg	tgt	ttc	atc	gtc	tcc	${\tt atc}$	ctc	atg	atc	ggc	2694
	Tyr		Asn	Gly	${\tt Trp}$	Ala		Phe	Ile	Val	Ser		Leu	Met	Ile	Gly	
251		795					800					805	***	~~~	+ = =	2.00	2742
253	cta	ctg	Thr	gct Mla	Dho	TIO	Clv	gac Acn	Lou	gct Ala	Cor	Uie	Dhe	Δla	Cve	Thr	2/42
255		цец	1111	ита	riie	815	GIY	мэр	nea	AIU	820	1113	I IIC	miu	CID	825	
		gcc	ctg	aag	gat		gtg	acc	gcg	gtg		ttc	gtt	gcg	ctt	gga	2790
258	Ile	Ala	Leu	Lys	Asp	Ser	Val	Thr	Ala	Val	Va1	Phe	Val	Ala		Gly	
259					830					835					840		
261	acc	tca	gtg	cca	gac	aca	ttt	gca	agc	aaa Lys	gtg	gcc	gcc	acc	cag	gac	2838
262	rnr	ser	vaı	Pro 845	Asp	rnr	rne	АТа	Ser 850	ьys	vai	ΗTG	wrq	855	GTIJ	vah	
	car	tat	aca		qca	tcc	ata	gat		gtc	aca	qqc	agc		qcq	gtg	2886
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#### Input Set : A:\Umo15311.app Output Set: N:\CRF3\07242001\I901419.raw

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266 Gln Tyr Ala Asp Ala Ser Ile Gly Asn Val Thr Gly Ser Asn Ala Val
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269 aac gtc ttc ctg ggc atc ggt gtg gcc tgg tcc atc gcc gcc atc tac
                                                                      2934
270 Asn Val Phe Leu Gly Ile Gly Val Ala Trp Ser Ile Ala Ala Ile Tyr
        875
271
273 cac gcg gcc aac ggg gaa cag ttc aaa gtg tcc cct ggc acg cta gct
                                                                       2982
274 His Ala Ala Asn Gly Glu Gln Phe Lys Val Ser Pro Gly Thr Leu Ala
                                            900
                        895
                                                                       3030
277 ttt. tet qtc act ctc ttc acc att ttt qct ttc atc aat qtg ggg gtg
278 Phe Ser Val Thr Leu Phe Thr Ile Phe Ala Phe Ile Asn Val Gly Val
279
                    910
                                        915
281 ctq ctq tat cqq cqq aqq cca qaa att gga ggt gag ctg ggt ggg ccc
282 Leu Leu Tyr Arg Arg Pro Glu Ile Gly Gly Glu Leu Gly Gly Pro
                925
                                    930
                                                                       3126
285 egg act gec aag etc etc aca tec tgc etc ttt gtg etc etg tgg etc
286 Arg Thr Ala Lys Leu Leu Thr Ser Cys Leu Phe Val Leu Leu Trp Leu
                                                    950
                                945
289 ttg tac att ttc ttc tcc tcc ctg gag gcc tac tgc cac ata aaa ggc
                                                                       3174
290 Leu Tyr Ile Phe Phe Ser Ser Leu Glu Ala Tyr Cys His Ile Lys Gly
                           960
293 ttc taa aggaacaatc agatgtagta aatttatata tatatacata tatatatata
                                                                      3230
294 Phe
295 970
297 cataaaaatt atgtataatg aacagaggaa actggcattt gtcatgtcca cccacctgct 3290
299 qatqqaatcc agcttcaaga gcagactctg tactagggcc ggagagagaa ggcatcacct 3350
301 cccgtttccc aggggcgttc gtcttgttga accaggcatg gaggcagggc catctttacg 3410
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305 ttgttttctg tttttgcttg ttttgggtcg gggtagggag gtggttgatg ttagggtttq 3530
307 gttttggttt tgcaggggga agatcagggt ttgtggtcct cttgtgggag gtgatgtcca 3590
309 atctcaatgg taaaaatgga aatcaggaag atgactctcc ctttgcccaa aaactttaaa 3650
311 aattattttq qaqtaaqaaa qgaaacgggc atggaagaag aaagaagcat gtcttcacca 3710
313 tattactaaa tttcatgcct tatctctgga gtgggagcag aggtgaagtc ctccctccaa 3770
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317 gtttaaagat acatcgctgc ctggcaccct tgttcaacag gtacaaaaac aacatgccta 3890
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321 ggccetgatt ctgatgttct acaccegetg attccccage ceteccatee caaacceett 4010
323 ctccggaccc tttacccctc gtacaaacag gaagaataac tccattcaaa aagcacacca 4070
325 teettteeat tegeate
                                                                      4087
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329 <211> LENGTH: 970
330 <212> TYPE: PRT
331 <213> ORGANISM: Bos taurus
333 <400> SEQUENCE: 2
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336 Ile Ala Met Val Ala Leu Leu Phe Ser His Val Asp His Ile Ser Ala
                2.0
                                     25
338 Glu Thr Glu Met Glu Gly Glu Gly Asn Glu Thr Gly Glu Cys Thr Gly
339
            35
                                 40
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VERIFICATION SUMMARY
PATENT APPLICATION: US/09/901,419

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Input Set : A:\Umo15311.app
Output Set: N:\CRF3\07242001\1901419.raw

L:10 M:270 C: Current Application Number differs, Replaced Application Number L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date